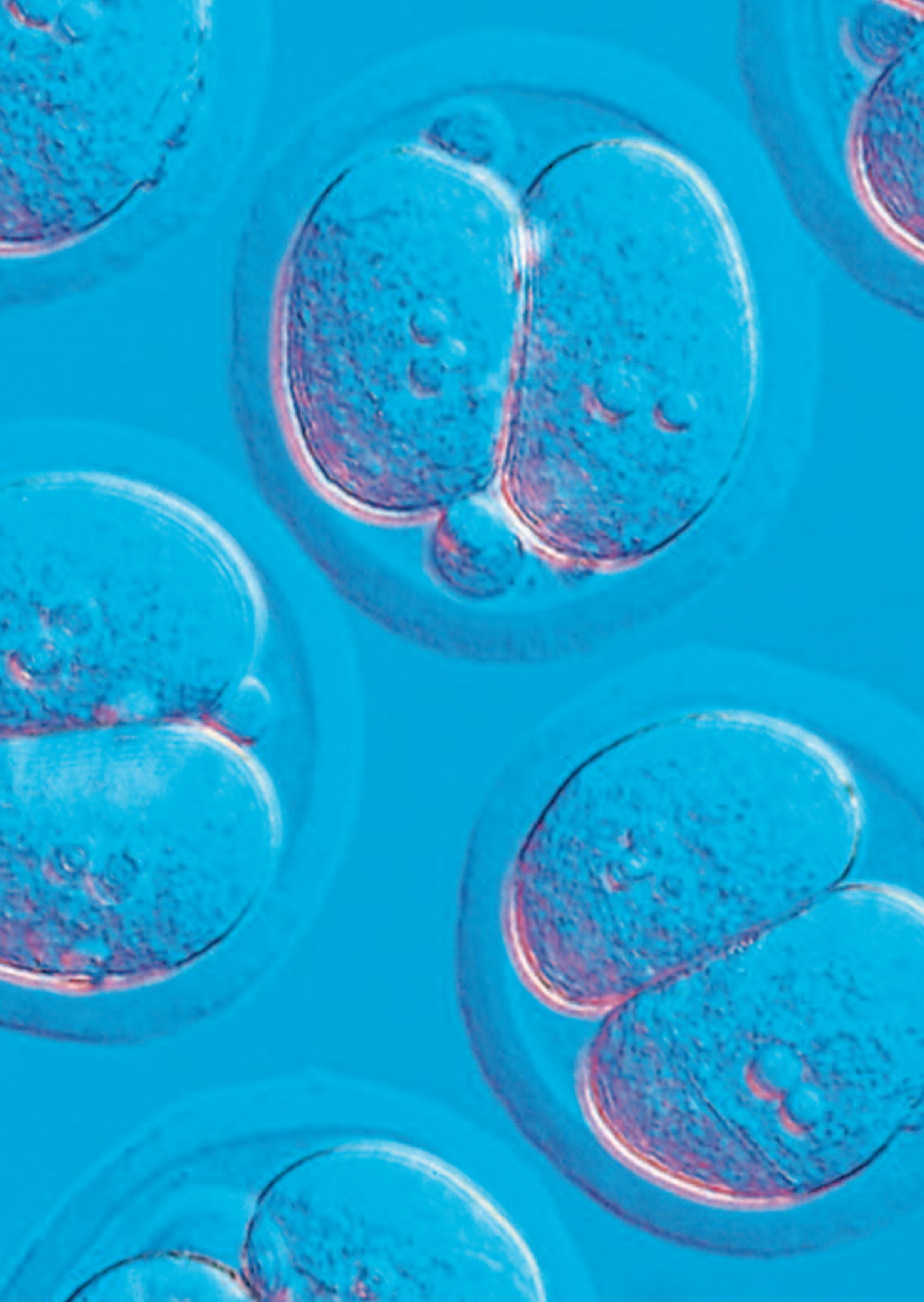
Start life with Nikon



Pioneers in IVF

Nikon microscopes have been a key part of developments in *in vitro* fertilisation (IVF) from the very start; the world's first IVF baby born in the UK and the first IVF baby in the US were conceived with the help of Nikon microscopes.

Since those pioneering days IVF has become a routine procedure. More than 34,000 women underwent IVF in the UK in 2006 and over 12,500 IVF babies were born as a result (Human Fertilisation and Embryo Authority data). In addition, a new generation of IVF techniques have emerged, such as ICSI (intracytoplasmic sperm injection), PGS/D (preimplantation genetic screening/diagnosis) and IMSI (intracytoplasmic morphology selected sperm injection). Nikon continues to be a leading developer and provider of microscopy and associated equipment in this important field.

Two core values are at the heart of Nikon's offering in IVF: a commitment to excellence in optical quality; and an ethos of developing cell-friendly imaging technologies. These are designed to minimise environmental stress and optimise cell viability during imaging, thereby providing embryos with the best possible start in life.

In this brochure, you will find information on Nikon IVF systems including upright microscopes, inverted systems for live cell imaging, stereo microscopes, micromanipulation equipment and the BioStation combined incubation and imaging system. You will also be able to find out how Nikon equipment has benefitted users in IVF. If you would like to find out more about Nikon systems for IVF and embryology, call Nikon today for information and advice tailored to your specific needs.

Developments in reproductive medicine

IVF, by definition, the fertilisation of eggs by sperm in the laboratory, usually involves four key steps: 1. inducing egg production and maturation in the woman with fertility hormones; 2. collecting the eggs and combining them with sperm in a culture dish; 3. monitoring fertilised eggs in the laboratory for several days (usually 2-3, but up to 5 days); and 4. implanting one or more of the most healthy embryos in the woman's uterus via a catheter. Unused eggs and embryos may be cryopreserved for future use.

IVF was originally used in women who had difficulty conceiving. Today it is relevant to almost all fertility problems in both men and women when combined with ICSI. ICSI accounts for over 40% of all IVF treatments in the UK.

Not every implanted embryo results in a live birth. Average success rates (using fresh eggs) range from 31% in women under 35 years of age to 4% in women over 44 years (HFEA: based on treatment carried out in 2006). Fertility specialists are faced with the dilemma of implanting sufficient embryos to increase the probability of success while at the same time avoiding risks associated with multiple pregnancies (premature birth, low birth weight and complications for both mother and baby). This also has to be weighed against the financial cost (between approx £4,000 and £8,000) and emotional stress of successive IVF cycles.

As pressure to reduce the number of multiple pregnancies in IVF increases, there is a greater need for strategies that will improve the chance of successful single embryo pregnancies. The importance of selecting only the most healthy eggs, sperm and embryos becomes paramount and microscopic imaging is an indispensible tool in this selection process. Embryo care during laboratory incubation also becomes more important to ensure embryo viability is not compromised before embryo transfer.

DID YOU KNOW?

The world's first 'test tube' baby, Louise Brown, was conceived with the help of a Nikon microscope.

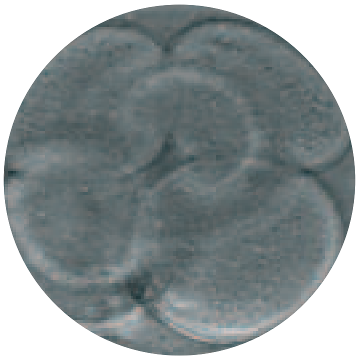
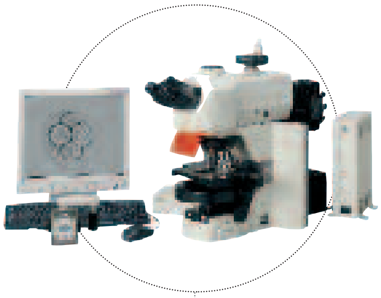
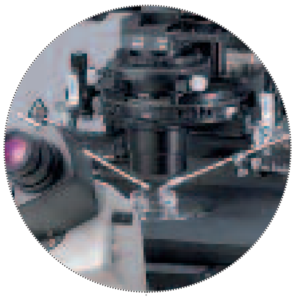


Image courtesy of Ronny Janssens, Centre for Reproductive Medicine, Brussels Free University, Belgium



90i

Andrology



Micro Manipulators

ICSI & PGD

SMZ 800, 1000 &1500

General embryology

Microscopic imaging: essential for IVF success

Clear high resolution microscopic imaging is essential for observing living sperm, eggs and embryos in culture at all stages of development. It is especially important for sperm and egg selection, monitoring embryo development, selecting healthy embryos for implantation and the delicate manipulation required in ICSI, PGD and embryo hatching procedures.

Upright microscopes: Nikon's i-series upright microscopes provide ideal systems for andrology. Acclaimed optics with high N.A.s and long working distances deliver superb images over the entire magnification range, ideal for sperm counting, motility and morphological studies.

Inverted microscopes: All Nikon's routine observation microscopes, from the TS100 series to the advanced Ti system, offer exceptional optical quality and robust, vibration-resistant design for IVF procedures. The Eclipse Ti is widely used for ICSI, IMSI, PGD and embryo hatching where high quality optics offer exquisite resolution of cell structure.

Multizoom microscope: Ideal for general embryology, the AZ100 Multizoom covers magnifications from 5x to 400x, combining the advantages of stereo zoom and compound microscopes to offer both macro and micro imaging in one instrument.

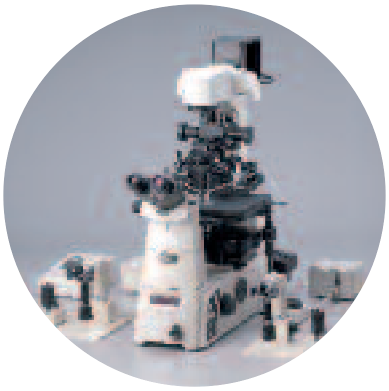
Stereo microscopes: The SMZ series of stereo microscopes offer zoom ratios from 6.3:1 to 15:1 with exceptional optical quality and vibration resistant, ergonomic design for a variety of embryology and IVF applications.

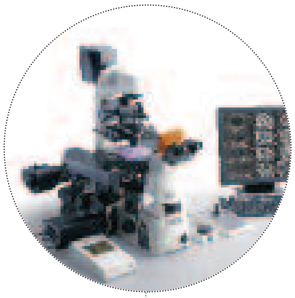
Micromanipulation equipment: To support IVF and embryology procedures choose from Nikon's extensive range of Narishige and Eppendorf products.

BioStation: For the ultimate solution in IVF imaging, Nikon's BioStation series allows monitoring and examination of embryos under perfectly controlled environmental conditions thereby reducing environmental stress and maximising embryo viability. BioStation's automated sample tracking and documentation system, in addition, provides an audit trail to GLP standards.

DID YOU KNOW?

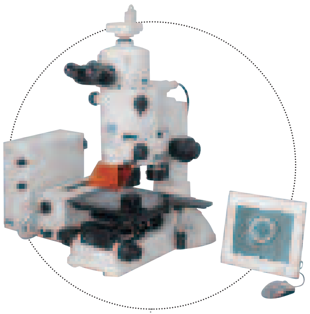
Nikon broke away from traditional microscope optical design in 1996 to create the revolutionary CFI60 infinity optical system. The result: long working distances, high N.A.s, and the ability to incorporate multiple imaging modules into the light path while maintaining optical quality.





Eclipse Ti

Live cell imaging & manipulation



AZ multizoom

Macro & micro in one

BioStation CT

Ultimate care for embryos

Critical selection decisions using the microscope

Eggs:

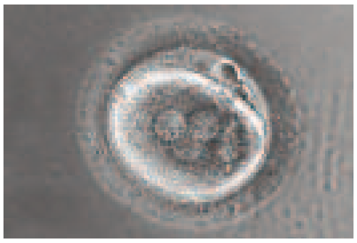
Following retrieval, eggs are usually examined under the microscope to select viable eggs for fertilisation. Morphological features that may be taken into account include size, degree of vacuolation, fragmentation and granulation, polar body and spindle morphology, integrity and thickness of the zona pellucida, and the presence and health of cumulus cells around the egg. The main criterion for selection is maturation as mature eggs are more likely to support normal embryo development. As many as 20% of eggs may be immature at the point of retrieval. These may be matured in the laboratory. It is even possible to mature eggs from unstimulated ovaries using the technique of IVM (*in vitro* maturation). IVM reduces the need for hormone treatment to obtain mature eggs and could be an alternative to conventional IVF, especially for women with polycystic ovaries.

DID YOU KNOW?

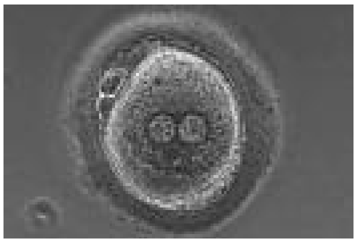
The first US test tube baby, Elizabeth Carr, was conceived with the help of a Nikon microscope

Sperm:

ICSI requires the selection of individual sperm for direct injection into eggs. Sperm quality may be evaluated microscopically through criteria such as maturity, sperm count, motility and morphology, especially of the head region containing the nucleus. Abnormal head shapes such as 'pin', 'amorphous', 'tapered', 'round' and 'multinucleated' are associated with reduced IVF success rates and can be identified with relatively low microscope magnification. However, even sperm appearing superficially normal may have subtle organelle defects that prevent fertilisation. These require magnifications of at least 6000x for detection (see IMSI). Other selection approaches might include the hypoosmotic swelling test to assess membrane integrity and the ability of sperm to bind to a gel that mimics the outer surface of the egg.



Abnormal embryo with three pro nuclei*



Normal embryo with two pro nuclei*

Embryos:

Embryo quality is generally assessed by cell number at defined time points, regularity of cells in terms of size and shape, and degree of cytoplasmic fragmentation. Other factors may include multinucleation, presence of vacuoles, granularity, and thickness of the zona pellucida. Decisions on which embryo(s) to implant are usually made on day three, although some laboratories, in some cases, maintain embryos *in vitro* for up to five days to aid the selection process.

*Images courtesy of: Ronny Janssens, Centre for Reproductive Medicine, Brussels Free University, Belgium

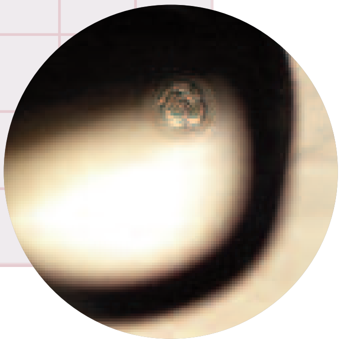
CASE STUDY: IVF WALES

Vitrification increases embryo survival and pregnancy rates

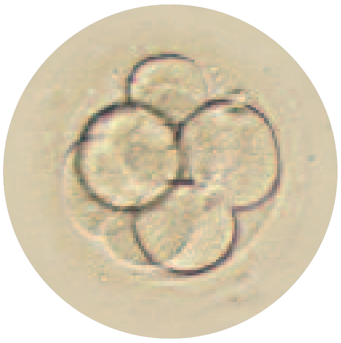
IVF Wales, at the University Hospital of Wales, Cardiff, was one of the first IVF clinics in the UK to use vitrification, a new cryopreservation technique. Because the cooling rate is about 20,000°C per minute, there is simply no time for potentially damaging ice crystals to form. "Since the introduction of the technique our pregnancy rate after cryopreservation has more than doubled compared to conventional freezing methods," says Mr Lyndon Miles, Head of Embryology and Andrology.

Embryos chosen for cryopreservation are selected on the same criteria as those for implantation. Microscopic examination is critical in this selection process. "I have been 'brought up' on Nikon microscopes having been trained and practised IVF techniques on Nikon/Narishige systems. We use a Nikon Eclipse TE2000S inverted microscope for embryo evaluation.

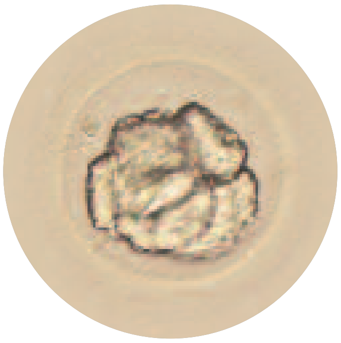
Most of our patients have embryo transfer on day three, and it is, therefore, on day three that critical selection decisions are made. As well as looking at the number of cells in the embryo, other factors such as fragmentation, vacuoles and embryo symmetry are taken into account. Environmental control on the microscope is critically important and there are many possible solutions from heated stages to microscope enclosures and microscopes contained within laminar flow hoods. Nikon's BioStation is undoubtedly the gold standard system for embryo observation as imaging is carried out automatically within a controlled, optimal incubator environment; embryos need never be taken out of the incubator for observation, thereby greatly reducing the risk of disturbance and environmental stress."



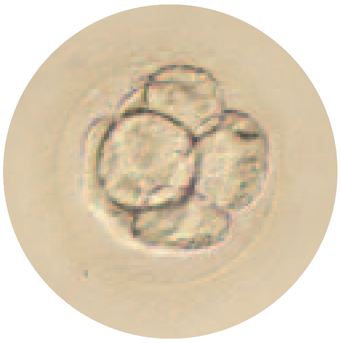
Embryo on CryoLeaf prior to plunging in liquid nitrogen*



Three day old embryo before vitrification*



Dehydrated embyo as water is taken from the embryo prior to vitrification*



Embryo five minutes after dehydration step prior to loading on cryoleaf*

*Images courtesy of: Mr Lyndon Miles, IVF Wales, Cardiff

IMSI

The aim in IMSI (Intracytoplasmic morphology selected sperm injection) is to select sperm with the greatest potential for success in ICSI. IMSI using magnifications of >6000x has resulted in higher pregnancy and delivery rates and lower abortion rates (Berkovitz et al, 2006 and 2005; Bartoov et al 2003).

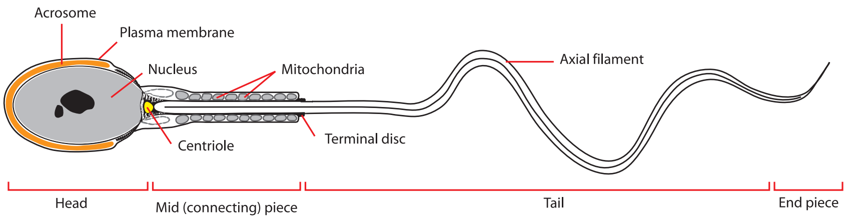
During IMSI, sperm are contained in a glass-bottomed dish under sterile paraffin oil with 4µl of sperm medium containing PVP (polyvinyl pyrrolidone) to reduce sperm motility. High magnifications enable detection of subtle defects in sperm organelles such as the acrosome, mitochondria, and nucleus. Acrosome abnormalities are a common cause of IVF failure as they prevent penetration of the egg by sperm. This cap-like structure on the sperm head contains lytic enzymes that digest the zona pellucida allowing sperm to inject haploid DNA into the egg. The post-acrosomal lamina, in addition, is the area of the sperm first recognised by the egg during fertilisation. This lines up internally with areas of the nucleus not covered by the acrosome. Defects in this structure may affect sperm-egg recognition and compromise fertilisation. Another common defect is abnormal vacuolation. Nuclear chromatin is considered abnormal if it contains more than one vacuole. The shape of the nucleus is also a key factor in deciding whether the sperm is normal as it determines the shape of the sperm head.

IMSI can be achieved on a Nikon inverted microscope equipped with high resolution Nomarski optics (100x or 60x oil Plan Apo VC) enhanced by a videozoom and digital imaging. The final magnification of the system can be calculated as follows:

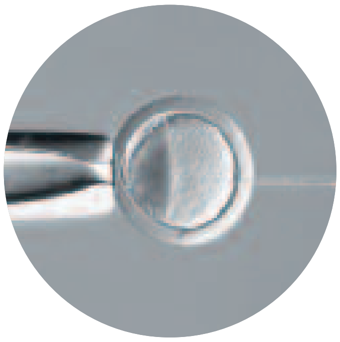
$$\textbf{(objective)}\ \text{X}\ \textbf{(magnification changer)}\ \text{X}\ \textbf{(videozoom)}\ \text{X}\ \frac{\textbf{(diagonal of image}}{\textbf{camera CCD chip diagonal)}}$$

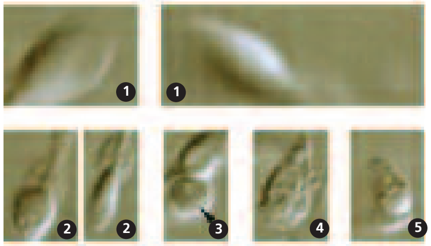
Being able to switch to DIC reveals details not visible with other contrast methods and provides greater imaging flexibility. Sperm examination normally uses 60x oil or 100x oil Plan Apo VC objectives followed by 20x or 40x non-oil objectives and Hoffman modulation contrast for ICSI. Users, therefore, have to use two different Petri dishes – the first with a 0.17mm glass bottom to select sperm using Plan Apo VC oil objectives and a second standard dish (for non-oil objectives) for ICSI. By using DIC for ICSI, rather than Hoffman modulation contrast, injection can be carried out with 20x or 40x oil objectives in the same glass-bottom Petri dishes.

The 60x oil objective is an ideal choice for sperm selection. As well as offering greater brightness and greater depth of focus than the100x objective, the 60x objective also provides a wider field-of-view. This is useful when observing rapidly-moving sperm, which may easily move out of a limited field-of-view.

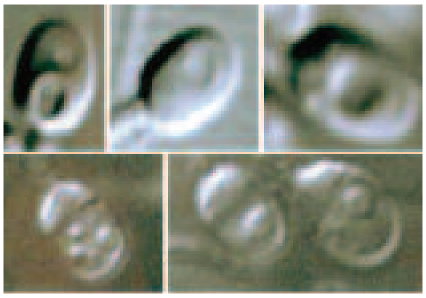


Regions of the normal sperm





1. Normal spermatozoa
2. Spermatozoa with excess residual cytoplasm
3. Microcephalic spermatozoa
4. Macrocephalic spermatozoa
5. Bent tail spermatozoa



Abnormal sperm showing vacuolisation observed with 100x oil immersion objective

References:

Berkovitz A et al (2006). Reprod Biomed Online;12(5):634-8.
Berkovitz A et al (2005). Hum Reprod;20(1):185-90.
Bartoov B et al (2003). Fertil Steril;80(6):1413-9.

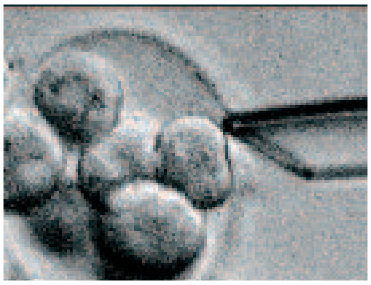
PGS/D (preimplantation genetic screening/diagnosis)

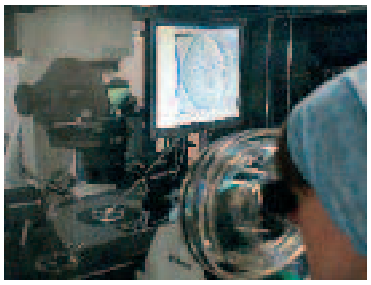
In addition to morphological assessment of sperm, eggs and embryos, PGS/D can help embryologists make informed decisions on which embryo(s) to select for implantation. It is often used when couples are affected by genetic disease or in cases where couples experience repeated, unexplained IVF failures.

PGS/D identifies chromosomal abnormalities using fluorescence *in situ* hybridisation (FISH), comparative genomic hybridisation (CGH) or PCR-based DNA amplification methods and is usually carried out on one or two cells taken from an embryo when it is around eight cells in size. It is a delicate procedure carried out under microscope observation. The embryo is positioned using a round ended micropipette and the zona pellucida perforated using a laser, enzymes or acid tyrode solution. A second pipette is then inserted into the embryo through the perforation to gently aspirate cells for analysis.

To determine abnormalities of maternal origin, PGS/D can also be applied to the first polar body of an egg or the second polar body formed shortly after an egg has been penetrated by sperm but before male and female DNA have combined.

DNA fragmentation in sperm is also associated with poorer IVF outcomes. Tests such as the comet assay, terminus dUTP nick-end labeling (TUNNEL) test, and sperm chromatin structure assays may have promise in helping to predict outcomes. The sperm mitochondrial genome is also considered a marker of sperm health.





Images courtesy of Dr Mary Herbert
Newcastle Fertility Centre at Life

CASE STUDY: THE FUTURE OF IVF (1)

The environment in which living cells are maintained is crucial to outcomes both in research and in the clinic. The Newcastle Fertility Centre for Life, in collaboration with Vitro Safe Systems™, has developed the first completely enclosed system for carrying out IVF procedures to avoid environmental stress during open manipulation of eggs and embryos, which could otherwise compromise results.

Dr Mary Herbert, Scientific Director of the Newcastle Fertility Centre. comments: “For years I have wanted to improve environmental control when working in IVF to protect embryos from temperature and pH fluctuations, which can compromise cell viability. Over a period of two years we have designed and built our Vitro Safe System™ for IVF. This is an enclosed, environmentally controlled system of eight workstations linked by seven integrated incubators with transfer hatches for consumables. Embryologists access samples and carry out procedures through a glove box system. “Nikon’s BioStation is of great interest to us as it provides the opportunity to automatically monitor embryos. Being able to access data on embryo developmental history would be a great help to compare embryos from the time of fertilisation to the transfer stage. Time to first cleavage, for example, is an important parameter. This would help us to select the best embryo from the cohorts of high quality embryos we are getting from our Vitro Safe™ system. My wish would be to incorporate automated live cell imaging using BioStation into the IVF flow system that we have.”

The importance of environment

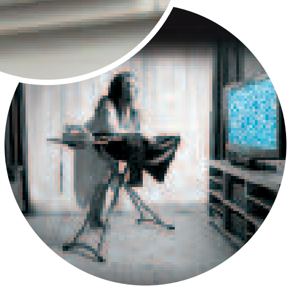
Whenever IVF samples are incubated, observed or manipulated it is essential that they are maintained in a contamination-free, optimal, cell-friendly environment that duplicates *in vivo* conditions as far as possible in terms of pH, temperature, gas concentration and humidity. Variations from the optimum can result in irreversible cell damage. Environmental control can be provided by a variety of means including stage top and/or microscope enclosures, enclosed environmentally controlled laminar flow hoods with 'glove box' access, and Nikon's BioStation.

Ideal for incubation and monitoring of IVF samples, Nikon's BioStation CT offers environmentally controlled incubation with integral automated microscope imaging according to user-programmed schedules. The system is ideal for embryo incubation, allowing embryologists to monitor development and assess embryo quality under stable, optimal conditions. Independent validation studies using eight autonomous sensors all around the inside of the incubator measuring CO_2, O_2, pH, humidity and temperature show a consistent environment even when the access gate is opened. A stable environment is vital to successful embryo culture and is a major step forward in standardising IVF procedures.

A complete environmental and imaging history is captured automatically for each embryo to provide an audit trail to GLP standards. Embryologists also have the benefit that they can monitor embryos remotely via the Internet or LAN at anytime and from anywhere – thereby allowing them to keep an eye on embryo status without having to be tied to the laboratory.

DID YOU KNOW?

The first successful ICSI procedure was carried out with the help of a Nikon microscope



CASE STUDY: THE FUTURE OF IVF (2)

The view that constant, controlled environments are critical to live cell imaging is shared by Karim Nayernia, Professor of Stem Cell Biology at Newcastle University. He also works in the Centre for Life and directs research that could have a major impact on male infertility. "Understanding how spermatogonial stem cells can give rise to pluripotent stem cells and how somatic stem cells differentiate into germ cells could give significant insight into the regulation of developmental totipotency as well as having important implications for male fertility and regenerative medicine," Professor Nayernia explains.

"When trying to investigate the effects of different growth factors on stem cell behaviour, environmental fluctuations can seriously compromise the interpretation of results. We now incubate and image cells inside BioStation which eliminates the need to remove cells for imaging. We can capture cell images as frequently as we like, even through the night, without incurring environmental stress on the cells or the need for anyone to remain in the laboratory. Cells can also be monitored remotely via the Internet or LAN, if needed."

Greater control over the environment in which cells and embryos are maintained promises more reliable and meaningful results from research; better outcomes for IVF; and new insights into the causes of infertility and congenital abnormalities. Ultimately, this will help in the development of a generation of new therapies, not only for infertility, but also tissue injury resulting from trauma, age-related degeneration and disease.

Supporting IVF

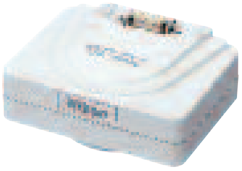
As well as its comprehensive range of microscopes for IVF, Nikon also supplies specialist objectives for live cell imaging, digital imaging equipment and image analysis software to enhance IVF imaging procedures.

DID YOU KNOW?

In the Nikon's BioStation embryos can be monitored without ever having to be taken out of the incubator.

Digital imaging

Nikon's digital cameras can be incorporated into IVF imaging systems to enable immediate image capture, archiving, the creation of an audit trail and immediate image sharing via a network. The DS-Qi1Mc camera is the camera of choice for IMSI because high sensitivity and fast capture rates are able to 'freeze' fast moving sperm. When using the 100x objective, the binning 2x2 function enables capture rates of 30 frames per sec. There are two options for videozoom, C-Mount Zooming Adapter Set (zooming range: 0.9 - 2.25x), or a fixed magnification 2.5x videozoom.



NIS-Elements

Nikon's intuitive NIS-Elements software simplifies workflow and speeds up image acquisition times while providing features such as image stitching, object counting and volume views. It is a powerful image management tool, which integrates cameras, components and peripherals with image archiving, analysis and visualisation tools.

Micromanipulators

Nikon supplies Narishige and Eppendorf micromanipulator equipment to support procedures such as ICSI, PGS/D and embryo hatching. The Narishige NT88-V3, for example, offers compact design to provide a reduced distance from manipulator mount to microelectrode tip thereby increasing stability. Eppendorf's TransferMan® NK 2 offers an excellent combination of automatic processes and intuitive operation to allow the most complex of micromanipulation techniques to be carried out rapidly and precisely. When combined with the FemtoJet and/or the CellTrams, the TransferMan NK 2 is ideal for injection into suspended cells.

Environmental enclosures

From heated stages (from Linkam and Tokai Hit) to full environmentally controlled microscope enclosures (from Okolab and Solent Scientific), Nikon supplies a variety of products for environmental control on the microscope.

BioStation

BioStation is the ultimate solution for embryo imaging under optimal environmental conditions. It eliminates the need to remove embryos for inspection and ensure that embryos develop in ideal conditions right up to the point of transfer.

Objectives

Long working distances are essential. Nikon's CFI60 optical system provides both long working distances as well as high N.A.s. Enabling high resolution observation of living unstained cells, apodized phase contrast objectives dramatically reduce halo effects compared with conventional lenses. A correction collar is also available for live cell work that compensates for the effects of working at physiological temperatures.

Let Nikon give your IVF programme a great start in life

email discover@nikoninstruments.eu

visit www.nikoninstruments.eu

Produced by Nikon (UK) Instruments